CLAIMS

What is claimed is:

1. A hyaluronic acid (HA) composition comprising crosslinked, water-insoluble, hydrated HA gel particles, wherein the HA includes crosslinks represented by the following structural formula:

$$HA'—U—R_2-U—HA'$$

wherein:

each HA' is the same or different crosslinked HA' molecule;

each U is independently an optionally substituted O-acyl isourea or N-acyl urea; and

R2 is optionally substituted alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, cycloalkenyl, cycloalkynyl aryl, heteroaryl, heterocyclyl, cycloaliphaticalkyl, aralkyl, heteroaralkyl, or heterocyclylalkyl.

2. The HA composition of Claim 1, wherein the particles include at least one bioactive agent selected from the group consisting of cells, genes, proteins, antibodies, peptides, and pharmaceuticals.

3. The HA composition of Claim 2, wherein the bioactive agent includes an anesthetic.

4. The HA composition of Claim 3, wherein the bioactive agent is lidocaine, mepivacaine, prilocaine, bupivacaine, cocaine, procaine, chlorocaine, or tetracaine, or a salt or solvate thereof.

5. The HA composition of Claim 4, wherein the bioactive agent is lidocaine·HCl.

6. The HA composition of Claim 1, wherein the particles have a particle average diameter distribution selected from the group consisting of:

a hydrated particle average diameter between about 20 μm and about 1000 μm; and

a dehydrated particle average diameter between about 10 μm and about 500 μm.

7. The HA composition of Claim 6, wherein the distribution is a multimodal distribution.

8. The HA composition of Claim 6, wherein the HA in the composition consists essentially of the crosslinked, water-insoluble, hydrated HA gel particles.

9. The HA composition of Claim 1, wherein the composition has at least one parameter measured at 37 °C selected from a storage modulus G' of at least about 50 Pa when measured at 1 Hz frequency using a 4 cm flat geometry, and a kinematic viscosity of at least about 20,000 cPs when measured at a shear rate of $1s^{-1}$.

10. The HA composition of Claim 9, whereupon combining the composition at 37 °C with hyaluronidase enzyme in an amount of about 0.3% by weight, under conditions suitable for reaction with hyaluronidase, the value of storage modulus G' for the composition measured after 16 hours of reaction is at least about 5% of the value of G' measured at less than about 15 min of reaction.

11. A method of augmenting tissue in a subject that is in need of tissue augmentation, comprising the steps of:

a) inserting a needle into a subject at a location in the subject that is in need of tissue augmentation, wherein the needle is coupled to a syringe loaded with a crosslinked HA composition that includes crosslinked, water-

insoluble, hydrated HA gel particles, wherein the HA includes crosslinks represented by the following structural formula:

$$HA'—U—R_2-U—HA'$$

wherein:

each HA' is the same or different crosslinked HA' molecule;

each U is independently an optionally substituted O-acyl isourea or N-acyl urea; and

R2 is optionally substituted alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, cycloalkenyl, cycloalkynyl aryl, heteroaryl, heterocyclyl, cycloaliphaticalkyl, aralkyl, heteroaralkyl, or heterocyclylalkyl, and

b) applying force to the syringe, whereby at least a portion of the HA composition is delivered into the subject.

12. The method of Claim 11, wherein the subject is human.

13. The method of Claim 12, wherein the particles include at least one bioactive agent selected from the group consisting of cells, genes, proteins, antibodies, peptides, and pharmaceuticals.

14. The method of Claim 13, wherein the bioactive agent includes a local anesthetic.

15. The method of Claim 14, wherein the bioactive agent is lidocaine, mepivacaine, prilocaine, bupivacaine, cocaine, procaine, chlorocaine, or tetracaine, or a salt or solvate thereof.

16. The method of Claim 15, wherein the bioactive agent is lidocaine·HCl.

17. The method of Claim 12, wherein the particles have an average particle diameter distribution selected from the group consisting of a hydrated particle average diameter between about 20 μm and about 1000 μm, and a dehydrated particle average diameter between about 10 μm and about 500 μm.

18. The method of Claim 17, wherein the distribution is a multimodal distribution.

19. The method of Claim 18, wherein the HA in the composition consists essentially of the crosslinked, water-insoluble, hydrated HA gel particles.

20. The method of Claim 12, wherein the composition has at least one parameter measured at 37 °C selected from a storage modulus G' of at least about 50 Pa when measured at 1 Hz frequency using a 4 cm flat geometry, and a kinematic viscosity of at least about 20,000 cPs when measured at a shear rate of $1s^{-1}$.

21. The method of Claim 20, wherein the composition has a storage modulus G' of at least about 100 Pa.

22. The method of Claim 21, wherein the composition has a storage modulus G' of at least about 400 Pa.

23. A method of preparing a hyaluronic acid (HA) composition, comprising the steps of:

a) forming water-insoluble, dehydrated crosslinked HA particles;

b) separating the water-insoluble, dehydrated particles by average diameter and selecting a subset of particles by average diameter;

c) hydrating the subset of dehydrated particles with a physiologically compatible aqueous solution, thereby forming the HA composition.

24. The method of Claim 23, further including hydrating a portion of the dehydrated particles having an average diameter between about 10 μm and about 1000 μm.

25. The method of Claim 24, further including the steps of:

d) separating the water-insoluble, dehydrated particles into at least three fractions by average diameter; and

e) selecting at least two average diameter fractions and rejecting at least one average diameter fraction.

26. The method of Claim 23, wherein the dehydrated particles are hydrated in the presence of the physiologically acceptable solution under conditions including a temperature of at least about 100 °C, a pressure of at least about 120 kPa, and a duration of at least about 15 min.

27. The method of Claim 23, wherein the physiologically compatible aqueous solution includes a bioactive agent.

28. The method of Claim 27, wherein the bioactive agent is lidocaine, mepivacaine, prilocaine, bupivacaine, cocaine, procaine, chlorocaine, or tetracaine, or salt or solvate thereof.

29. The method of Claim 28, wherein the bioactive agent is lidocaine·HCl.

30. The method of Claim 23, further including preparing the dehydrated, crosslinked HA by:

a) crosslinking a precursor of the crosslinked HA with a biscarbodiimide in the presence of a pH buffer that is at a pH between about 4 and about 8, wherein the resulting crosslinked HA includes crosslinks represented by the following structural formula:

$HA'—U—R_2-U—HA'$

wherein:

each HA' is the same or different crosslinked HA' molecule;

each U is independently an optionally substituted O-acyl isourea or N-acyl urea; and

R2 is optionally substituted alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, cycloalkenyl, cycloalkynyl aryl, heteroaryl, heterocyclyl, cycloaliphaticalkyl, aralkyl, heteroaralkyl, or heterocyclylalkyl, and

b) dehydrating the crosslinked HA to produce the dehydrated, crosslinked HA.

31. The method of Claim 30, wherein the pH is about 5.5.

32. A method of preparing a crosslinked hyaluronic acid (HA) composition, comprising the steps of:

a) crosslinking a precursor of the crosslinked HA with a biscarbodiimide in the presence of a pH buffer that is at a pH between about 4 and about 8, wherein the resulting crosslinked HA includes crosslinks represented by the following structural formula:

$HA'—U—R_2-U—HA'$

wherein:

each HA' is the same or different crosslinked HA' molecule;

each U is independently an optionally substituted O-acyl isourea or N-acyl urea; and

R2 is optionally substituted alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, cycloalkenyl, cycloalkynyl aryl, heteroaryl, heterocyclyl, cycloaliphaticalkyl, aralkyl, heteroaralkyl, or heterocyclylalkyl, and

b) dehydrating the crosslinked HA to produce the dehydrated, crosslinked HA.

33. The method of Claim 32, wherein the buffer includes at least one buffer agent selected from the group consisting of 2-(N-morpholino)ethanesulfonic acid; 2,2-bis(hydroxymethyl)-2,2',2"-nitrotriethanol; succinate/succinic; KH_2PO_4; N-tris(hydroxymethyl-2-aminoethanesulfonic acid; triethanolamine; diethylbarbituate; tris(hydroxymethyl)aminoethane; N-tris(hydroxy)methylglycine; and N,N-bis(2-hydroxyethyl)glycine.

34. The method of Claim 32, wherein the biscarbodiimide is at least one member selected from the group consisting of 1,6-hexamethylene bis(ethylcarbodiimide), 1,8-octamethylene bis(ethylcarbodiimide), 1,10 decamethylene bis(ethylcarbodiimide), 1,12 dodecamethylene bis(ethylcarbodiimide), PEG-bis(propyl(ethylcarbodiimide)), 2,2'-dithioethyl bis(ethylcarbodiimde), 1,1'-dithio-p-phenylene bis(ethylcarbodiimide); *para*-phenylene-bis(ethylcarbodiimide), and 1,1'-dithio-m-phenylene bis(ethylcarbodiimide).

35. The method of Claim 32, further including the steps of:

c) dehydrating the crosslinked HA to form dehydrated HA;

d) grinding the dehydrated HA to form water-insoluble, dehydrated HA particles;

e) separating the water-insoluble, dehydrated particles by average diameter and selecting a subset of particles by average diameter; and

f) hydrating the subset of dehydrated particles with a physiologically compatible aqueous solution, thereby forming the HA composition.

36. The method of Claim 35, wherein the dehydrated particles are hydrated in the presence of the physiologically acceptable solution under conditions including a

temperature of at least about 100 °C, a pressure of at least about 120 kPa, and a duration of at least about 15 min.

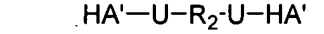
37. The method of Claim 36, wherein the pH is about 5.5.

38. A stabilized hyaluronic acid (HA) composition comprising crosslinked HA and at least about 0.1% by weight of a local anesthetic, wherein the value of storage modulus G' for the stabilized composition is at least about 110% of the value of G' measured for a non-stabilized composition, when measured at 37 °C and 1 Hz frequency using a 4 cm flat geometry.

39. The composition of Claim 38, wherein the value of G' for the stabilized composition is at least about 150% of the value of G' for the non-stabilized composition.

40. The composition of Claim 39, wherein the local anesthetic is lidocaine·HCl.

41. The composition of Claim 40, wherein the HA composition includes crosslinked, water-insoluble, hydrated HA gel particles, wherein the particles include crosslinks represented by the following structural formula:



wherein:

each HA' is the same or different crosslinked HA' molecule;

each U is independently an optionally substituted O-acyl isourea or N-acyl urea; and

R2 is optionally substituted alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, cycloalkenyl, cycloalkynyl aryl, heteroaryl, heterocyclyl, cycloaliphaticalkyl, aralkyl, heteroaralkyl, or heterocyclylalkyl.

42. A method of stabilizing crosslinked hyaluronic acid (HA), comprising hydrating water-insoluble, dehydrated crosslinked HA with a physiologically compatible aqueous solution, thereby forming the stabilized HA composition, wherein the physiologically compatible aqueous solution includes at least about 0.1% by weight of a local anesthetic, wherein the value of storage modulus G' for the stabilized composition is at least about 110% of the value of G' measured for a non-stabilized composition, when measured at 37 °C and 1 Hz frequency using a 4 cm flat geometry.

43. The method of Claim 42, wherein the dehydrated crosslinked HA is hydrated in the presence of the physiologically acceptable solution under conditions including a temperature of at least about 100 °C, a pressure of at least about 120 kPa, and a duration of at least about 15 min.

44. The composition of Claim 43 wherein the value of G' for the stabilized composition is at least about 150% of the value of G' for the non-stabilized composition.

45. The composition of Claim 44, wherein the local anesthetic is lidocaine·HCl.

46. The composition of Claim 45, wherein the HA composition includes crosslinked, water-insoluble, hydrated HA gel particles, wherein the particles include crosslinks represented by the following structural formula:

$$HA'—U—R_2-U—HA'$$

wherein:

each HA' is the same or different crosslinked HA' molecule;

each U is independently an optionally substituted O-acyl isourea or N-acyl urea; and

R2 is optionally substituted alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, cycloalkenyl, cycloalkynyl aryl, heteroaryl, heterocyclyl, cycloaliphaticalkyl, aralkyl, heteroaralkyl, or heterocyclylalkyl.

47. A hyaluronic acid (HA) composition comprising crosslinked, water-insoluble, hydrated HA gel particles, wherein:

a) the particles include lidocaine·HCl;

b) the particles have an average diameter selected from the group consisting of a hydrated particle average diameter between about 20 and about 1000 μm, and a dehydrated particle average diameter between about 10 and about 500 μm;

c) the particles include crosslinks represented by the following structural formula:

$$HA'—U—R_2-U—HA'$$

wherein:

each HA' is the same or different crosslinked HA' molecule;

each U is independently an optionally substituted O-acyl isourea or N-acyl urea; and

R2 is optionally substituted alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, cycloalkenyl, cycloalkynyl aryl, heteroaryl, heterocyclyl, cycloaliphaticalkyl, aralkyl, heteroaralkyl, or heterocyclylalkyl, and

d) the composition has at least one parameter measured at 37 °C selected from a storage modulus G' of at least about 50 Pa when measured at 1 Hz frequency using a 4 cm flat geometry, and a kinematic viscosity of at least about 20,000 cPs when measured at a shear rate of $1s^{-1}$; and

e) the composition is sufficiently stable to enzymatic degradation that upon combining the composition at 37 °C with hyaluronidase enzyme in an

amount of about 0.3% by weight, under conditions suitable for reaction with hyaluronidase, the value of G' for the composition measured after 16 hours of reaction is at least about 5% of the value of G' measured at less than about 15 min of reaction.

5

48. The HA composition of Claim 47, wherein the value of G' for the composition measured after 16 hours of reaction is at least about 50% of the value of G' measured at less than about 15 min of reaction.